Tango-Workshop 4. - 6. März 2015
Scientific Intro: BioDiff - a Diffractometer Optimized for Crystals with Large Unit Cells

T. E. Schrader

Jülich Centre for Neutron Science, Outstation at MLZ

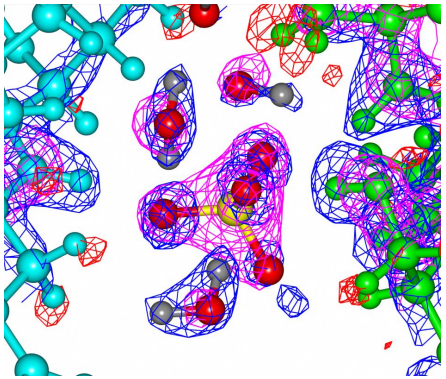
Advantages of Structure Determination with Neutrons

Hydrogen/deuterium atoms can be resolved even at a resolution of $d_{min} \approx 2.5$ Å (for ^2H). Therefore one can determine:

→ protonation states of amino acid side chains and ligands

→ deuterium exchange as a measure of flexibility and accessibility (discrimination between **H** / **D**)

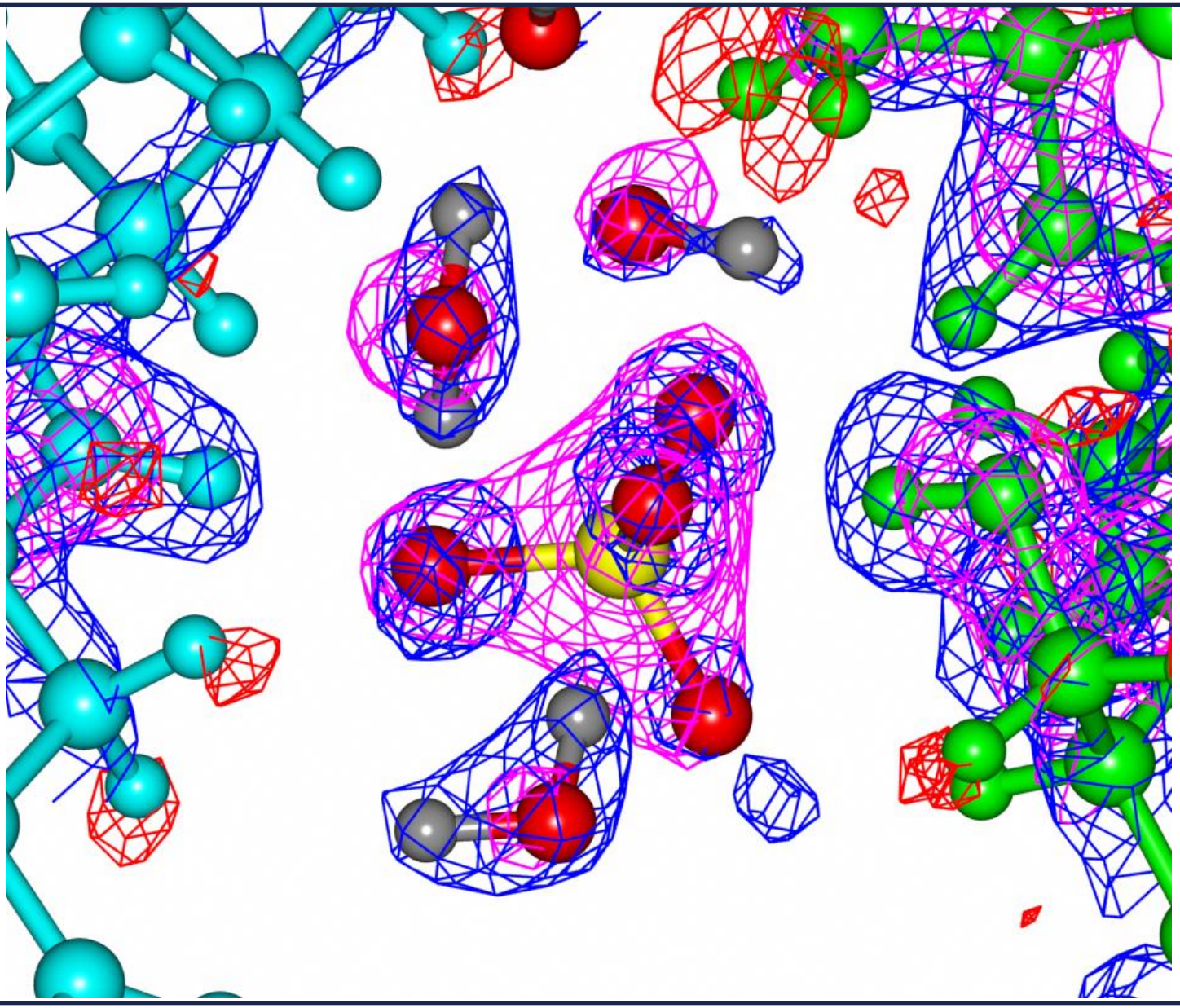
→ solvent structure including hydrogen atoms



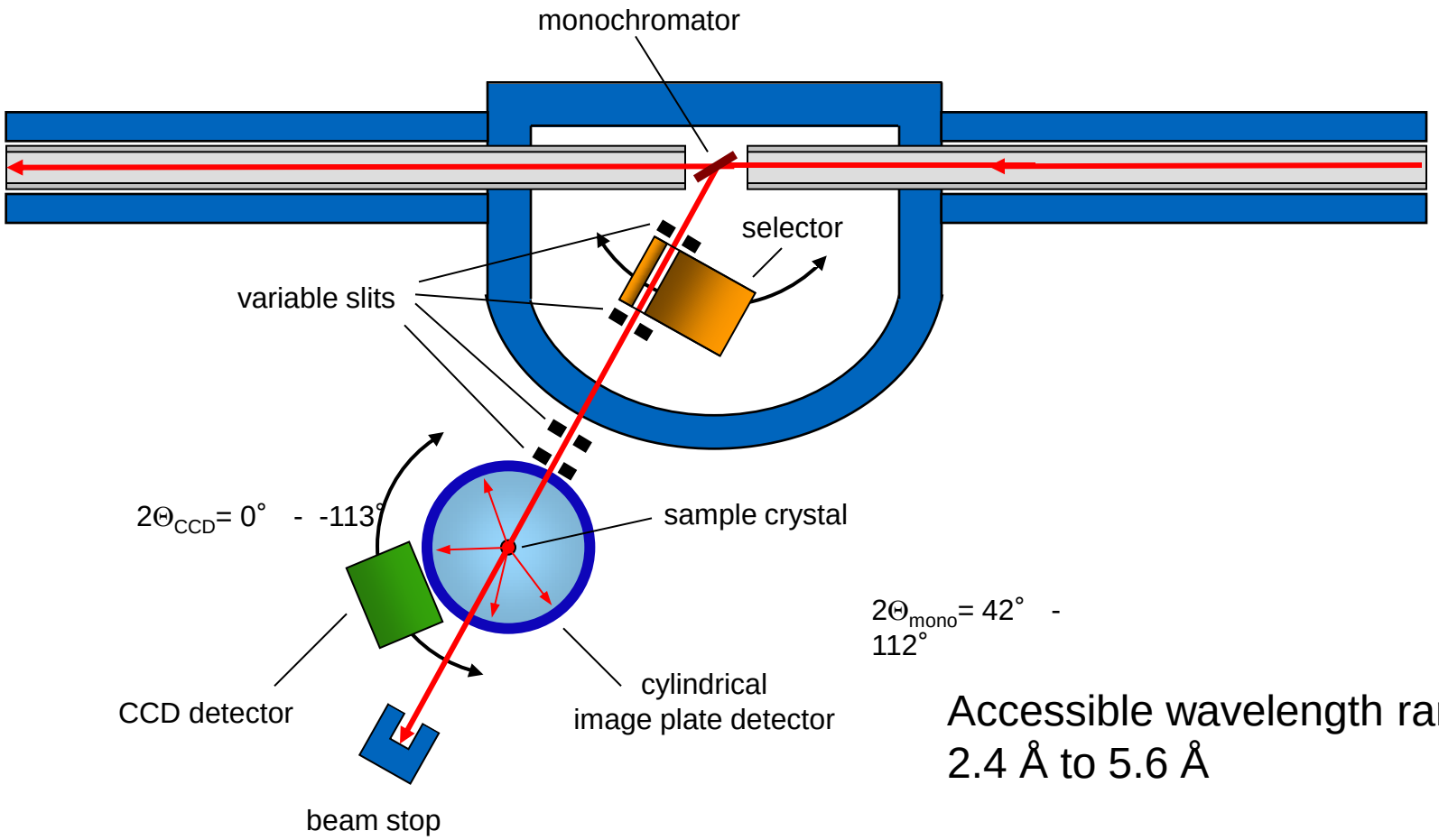
Water network in the contact region between two myoglobin molecules in the crystal.

x-ray map (magenta): contour level of +2.7σ
nuclear map (red): contour level of -1.75σ
nuclear map (blue): contour level of +2.3σ

Much less radiation damage as compared to x-rays: Metallo-proteins can be measured without reducing the metal centres

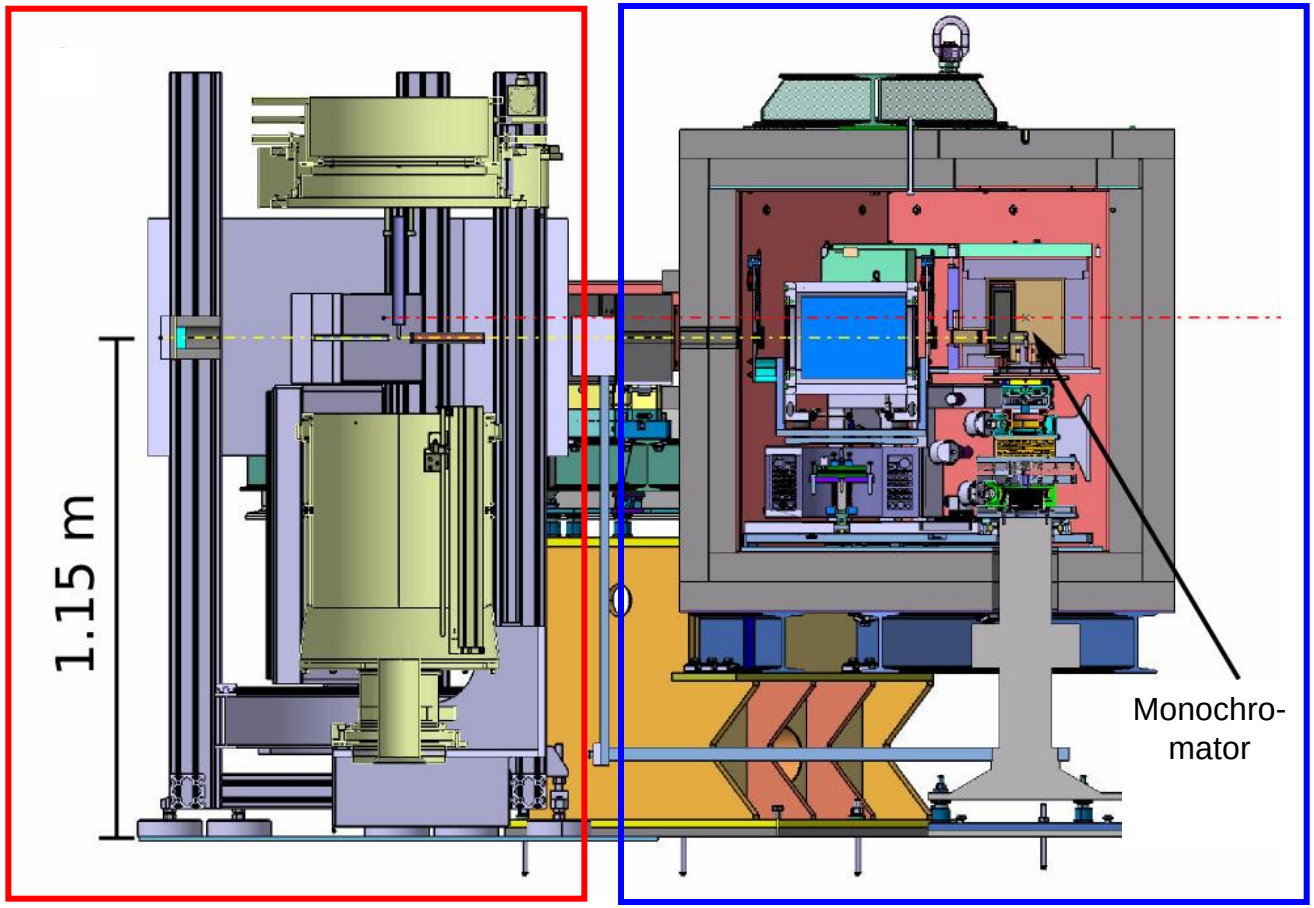


Schematic Overview over the Instrument BioDiff



Accessible wavelength range:
2.4 Å to 5.6 Å

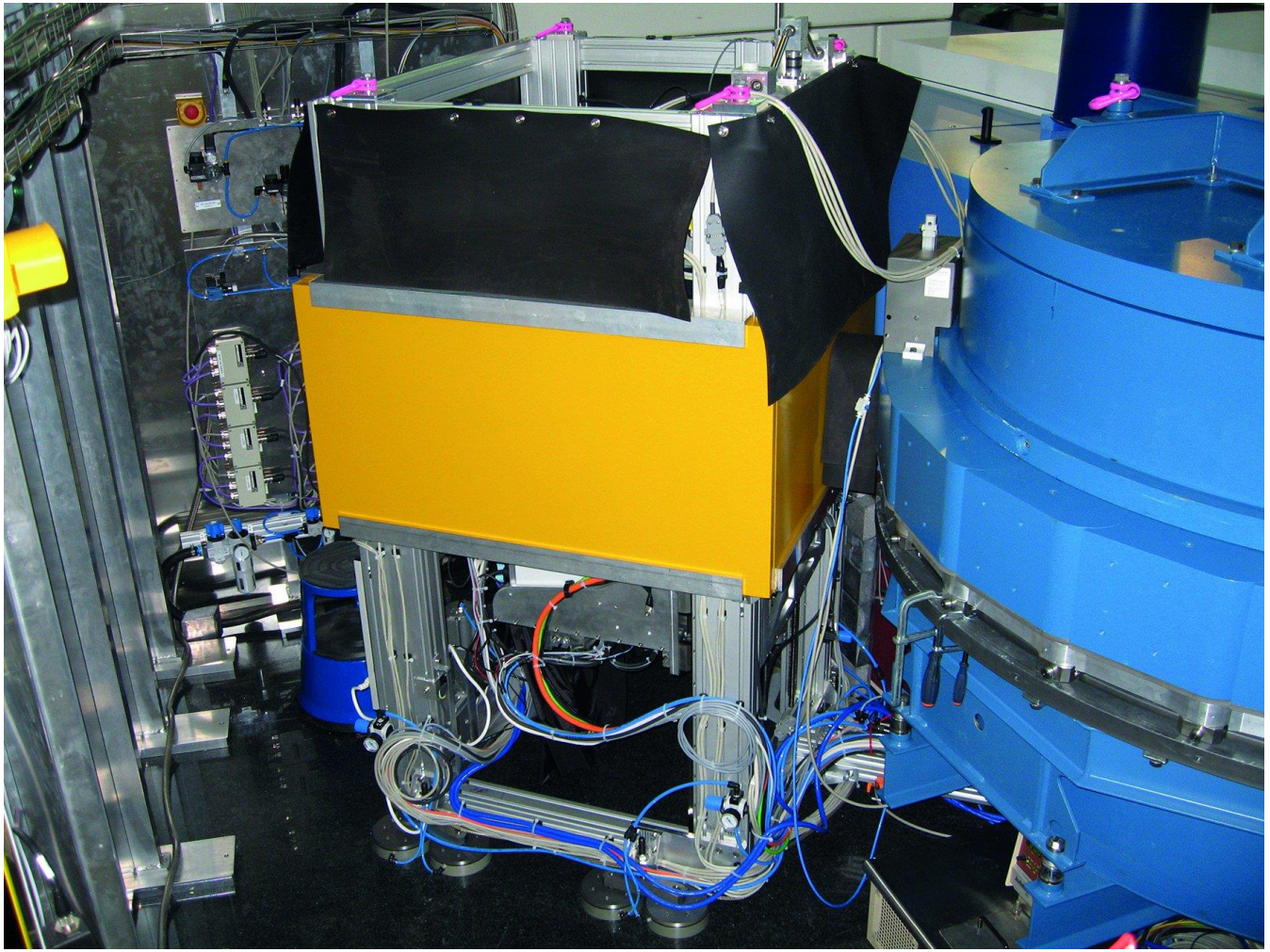
The Simultaneous Construction-phase in Garching and Jülich



Detector unit, constructed and built in Garching (Ph. Jüttner, MLZ)

Monochromator-shielding, constructed and built in Juelich (B. Laatsch, ZEA-1 Engineering)

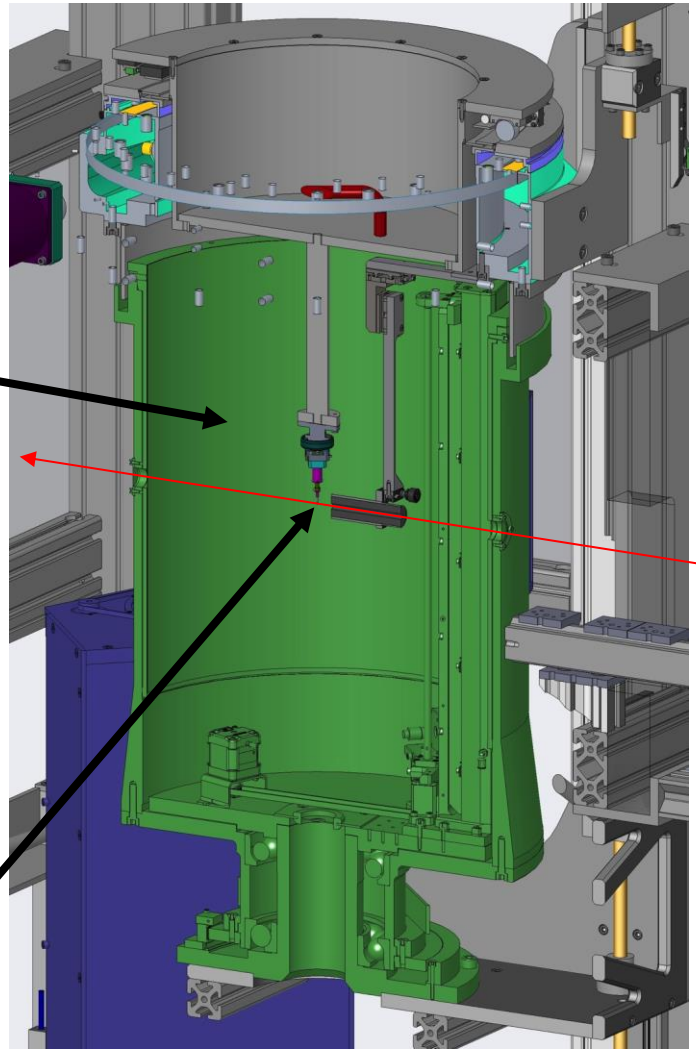
A Most Recent View of the Instrument BioDiff



Detector Unit: Neutron Image Plate

neutron image plate

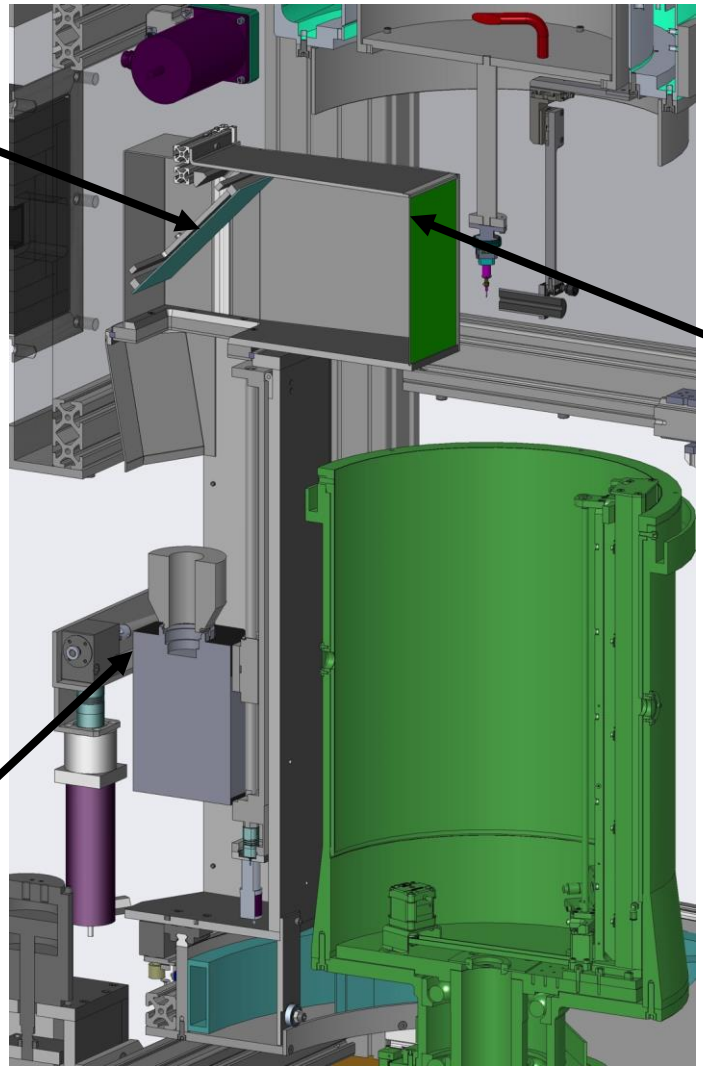
- Gd_2O_3 / $BaFBr:Eu^{2+}$ (white Niimura-type)
- cylindrical shape: r = 200mm; h = 450mm
- scanner resolution: 125, 250, 500 μm
- readout time + erasing: ≈ 4min (500 μm)

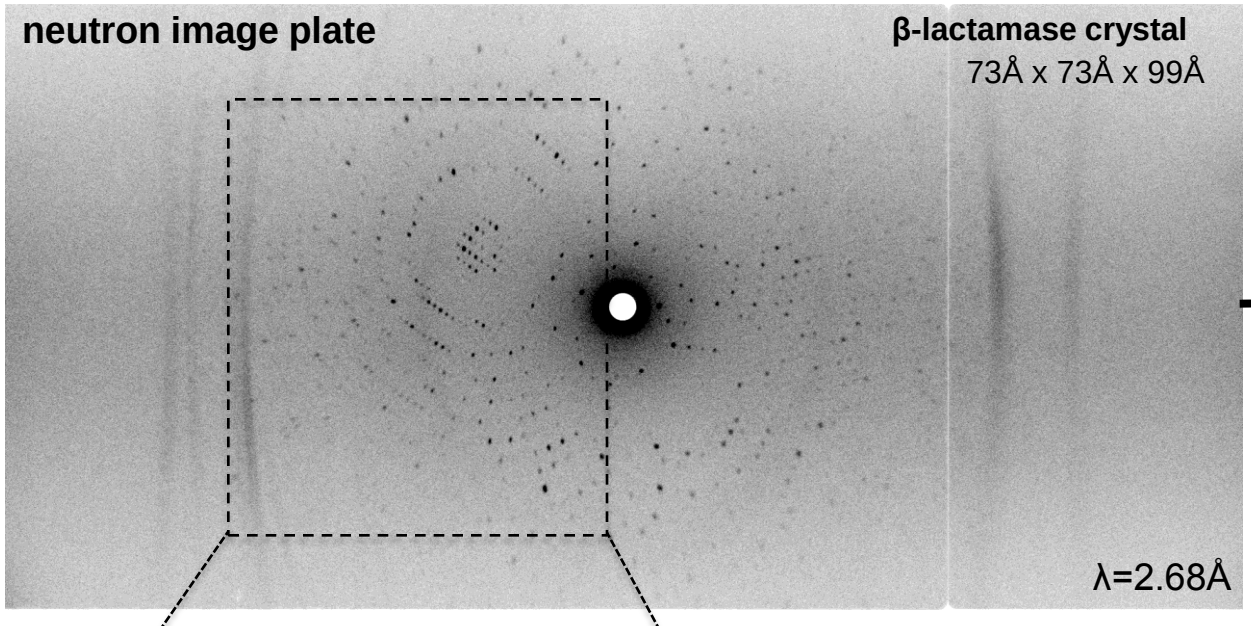


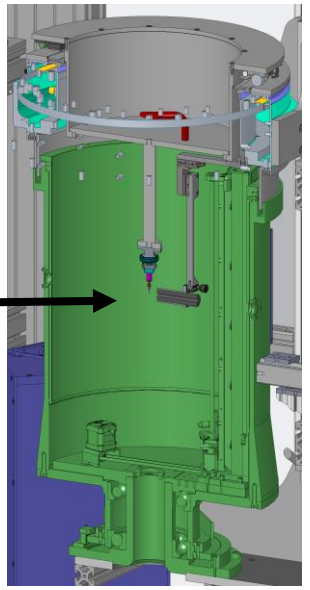
Detector Unit: CCD-camera

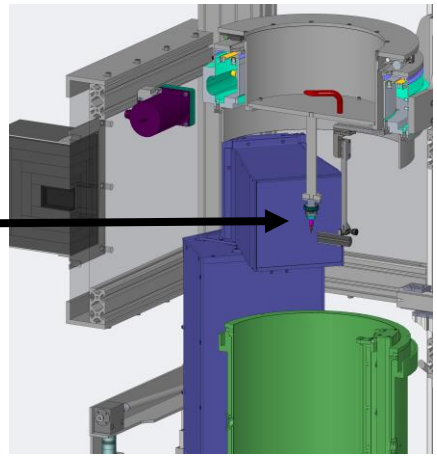
Scintillator based CCD-camera

- ^{6}LiF/ZnS scintillator
- flat shape: 200 x 200mm
- overall resolution: ≈300 μm
- Andor iKon-L CCD camera
- readout time: ≥1sec









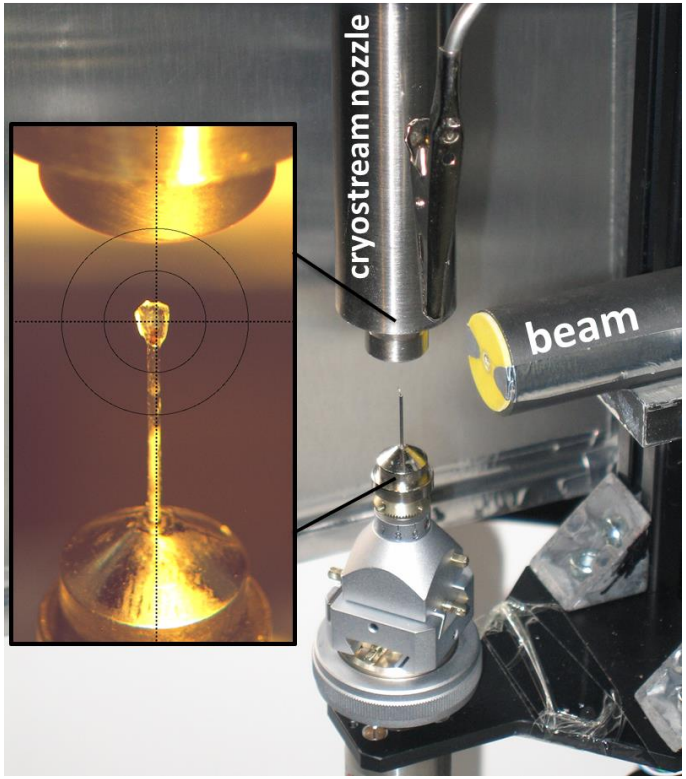
NIP-scanner

- larger covered solid angle
- readout time ≥ 4min

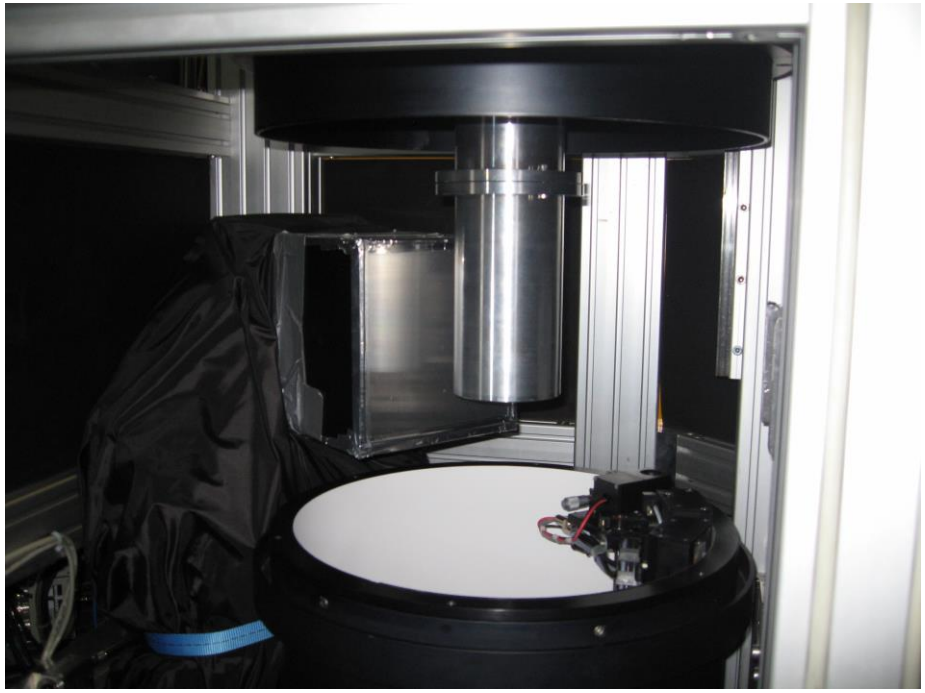
CCD-camera

- smaller covered solid angle
- readout time ≥ 1sec

Available Sample Environment at BioDiff



BioDiff with mounted cryostream sample environment allowing for temperatures between 90 K and 500 K

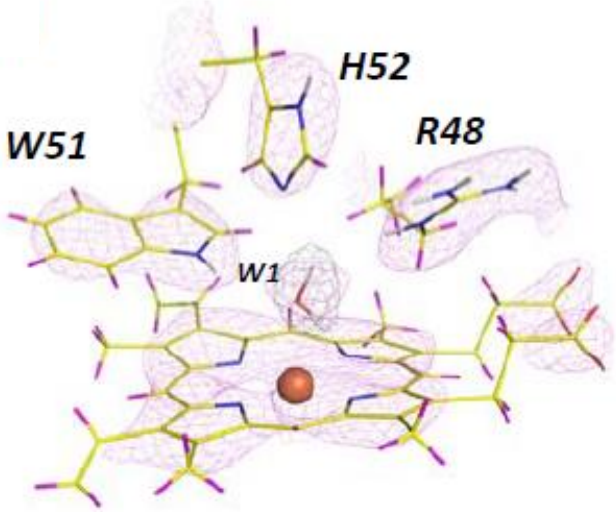


Closed cycle cryostat for temperatures down to 3.5 K

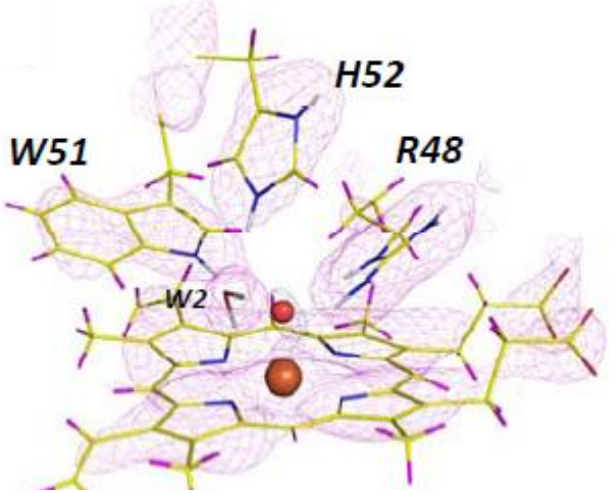
Leighton Coates, Stephen Tomanicek, Tobias Schrader, Kevin Weiss, Joseph Ng, Philipp Juttner and Andreas Ostermann, Journal: Journal of Applied Crystallography, accepted

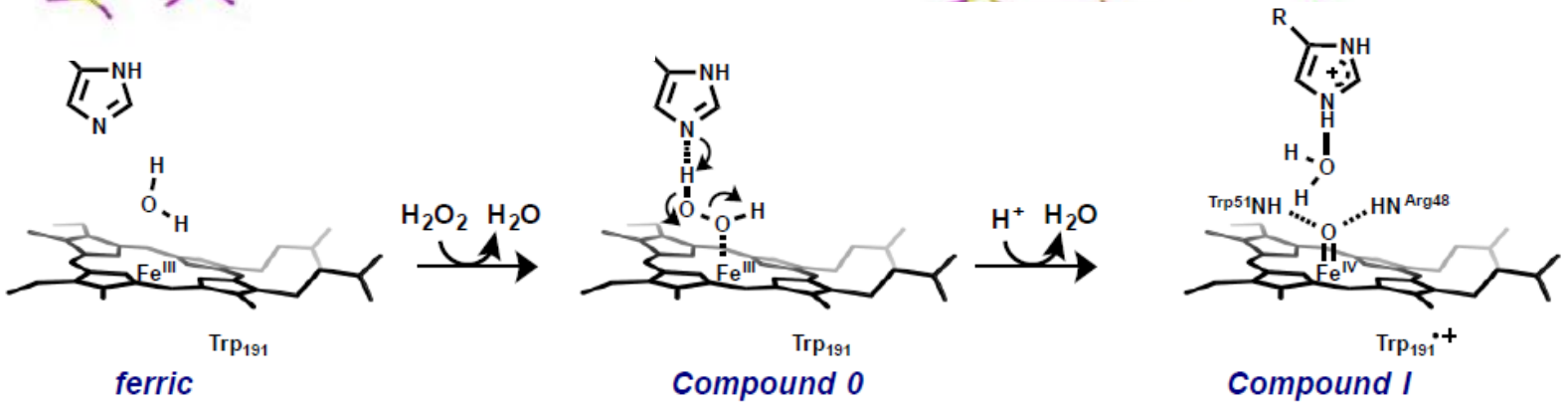
Application example: The protonation state of ferryl heme in a peroxidase

LADI-III at ILL, room temperature



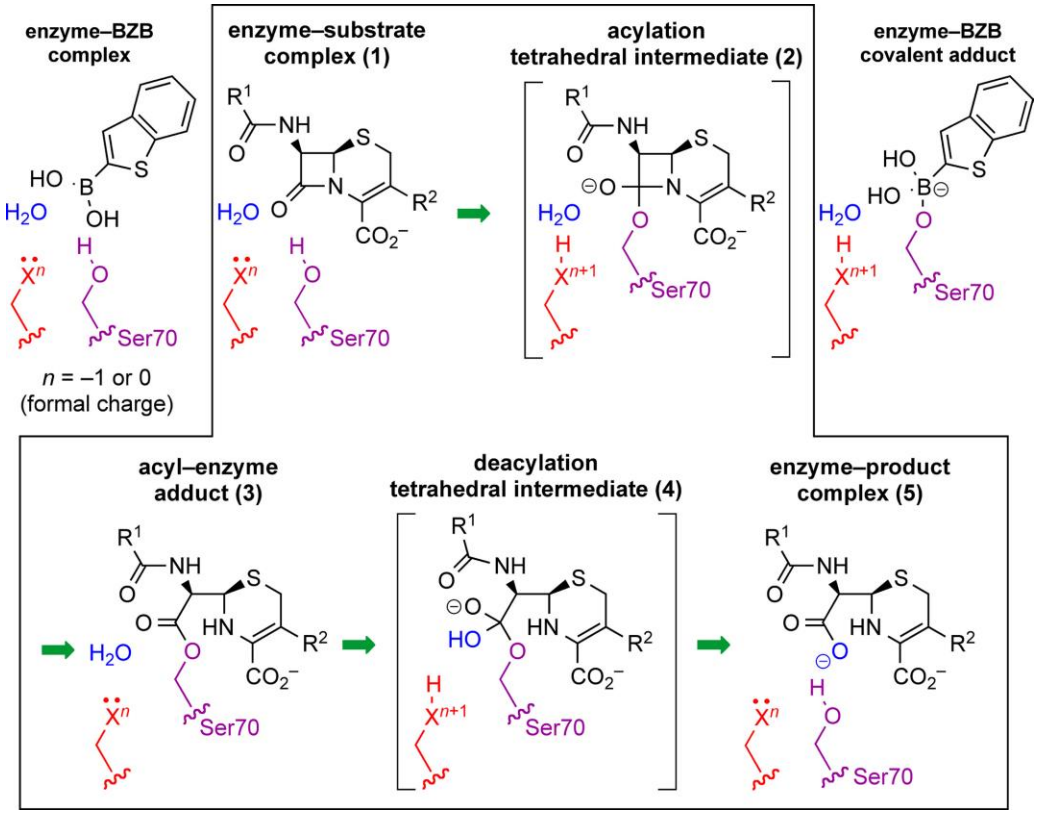
BioDiff at MLZ, 100 K





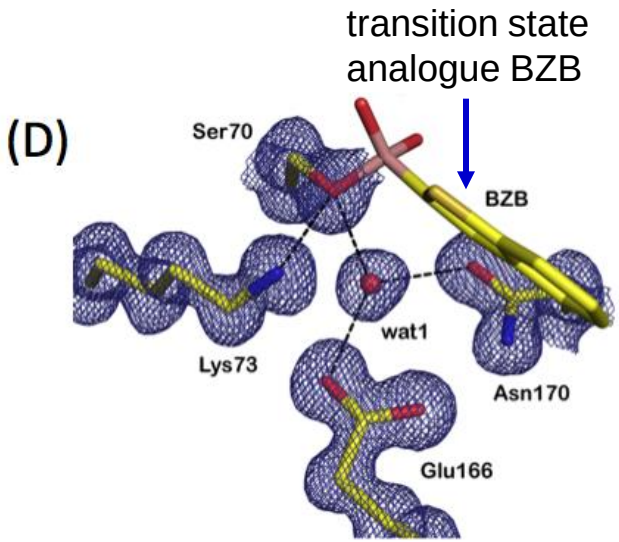
Experimental team: Cecilia M. Casadeia, Andrea Gumieroc, Clive L. Metcalfe, Emma J. Murphy, Jaswir Basran, Susana C. M. Teixeira, Maria Grazia Concilio, Tobias E. Schrader, Alistair J. Fielding, Andreas Ostermann, Matthew P. Blakeley, Emma L. Raven & Peter C. E. Moody, paper submitted

Catalytic proton network of the Toho-1 β-lactamase

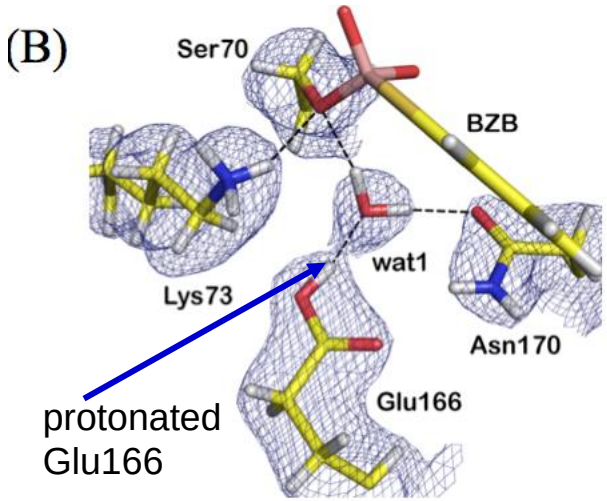


The catalytic cycle of a class A-lactamase illustrated for a cephalosporin substrate (*inside box*) and the mode of inhibition by BZB (*outside box*). The general base employed is not necessarily the same for acylation and deacylation.

Application Example:
Catalytic Proton Network of the Toho-1 β-Lactamase



(D) electron density map

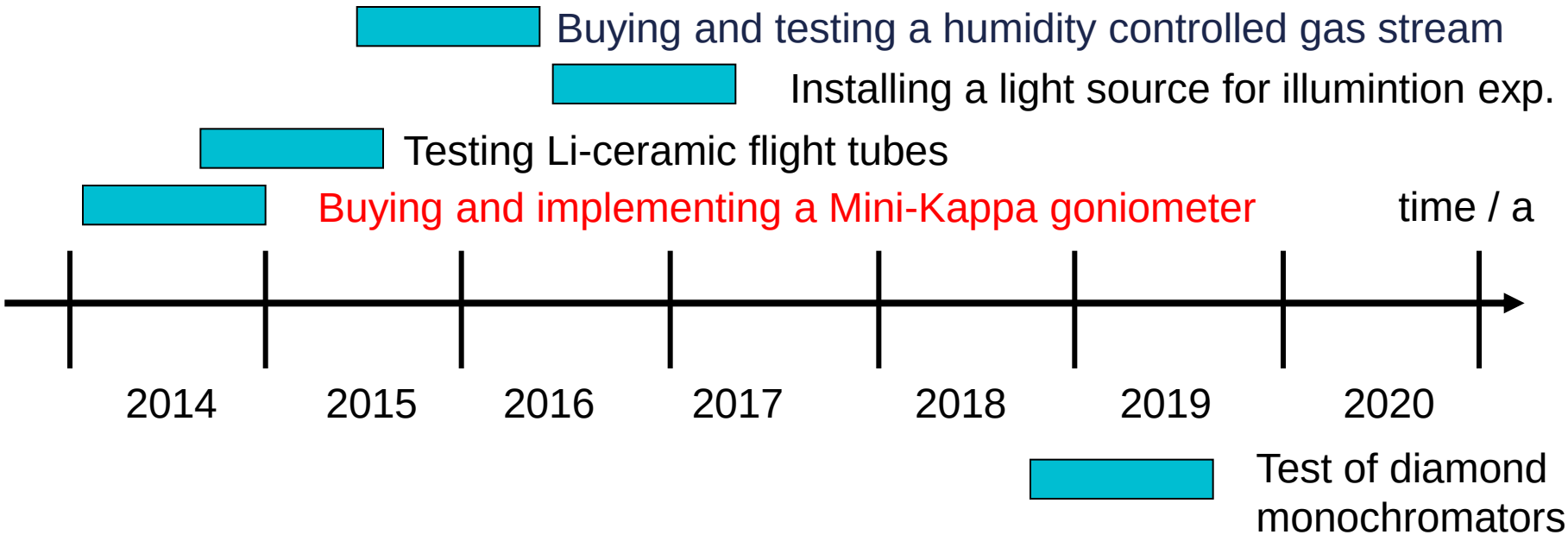


(B) nuclear density map from BioDiff

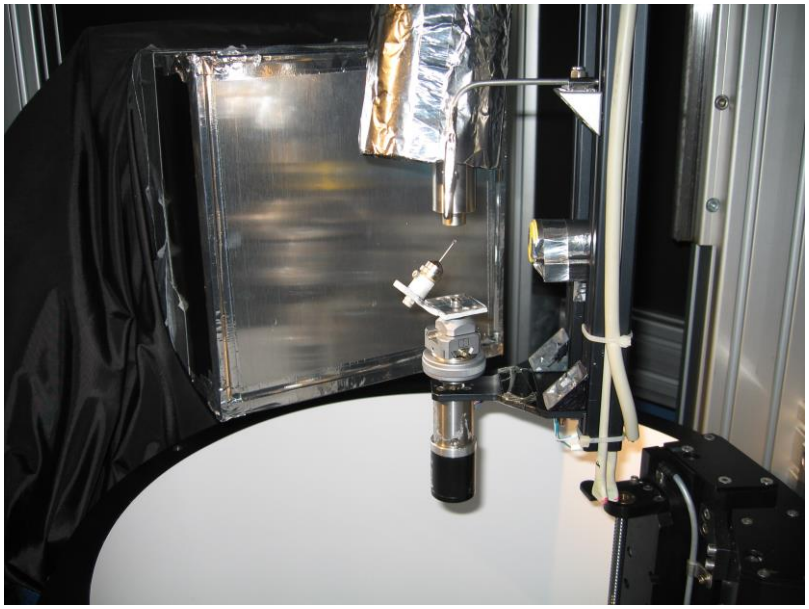
Glu166 acts as the general base during the catalytic action of the enzyme.

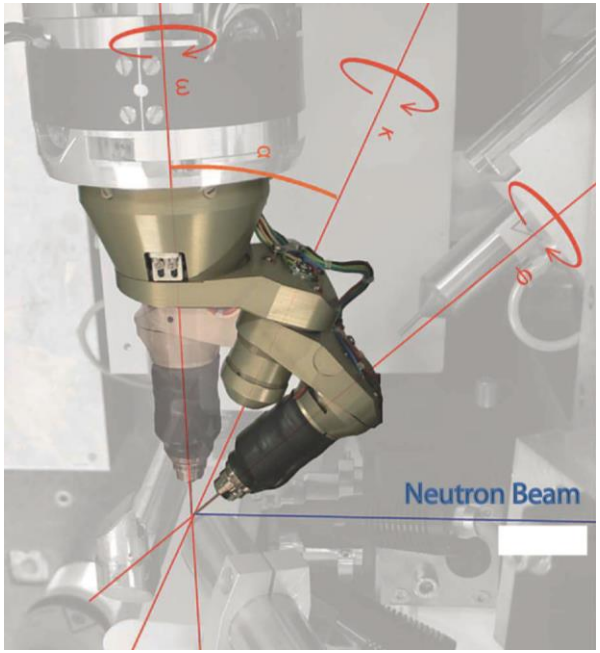
Stephen J. Tomanicek, Robert F. Standaert, Kevin L. Weiss,
Andreas Ostermann, Tobias E. Schrader, Joseph D. Ng, and Leighton Coates
J. Biol. Chem. 2013, 288:4715-4722

The Instrument Development Programme: Time Schedule



Mini-Kappa Goniometer Head

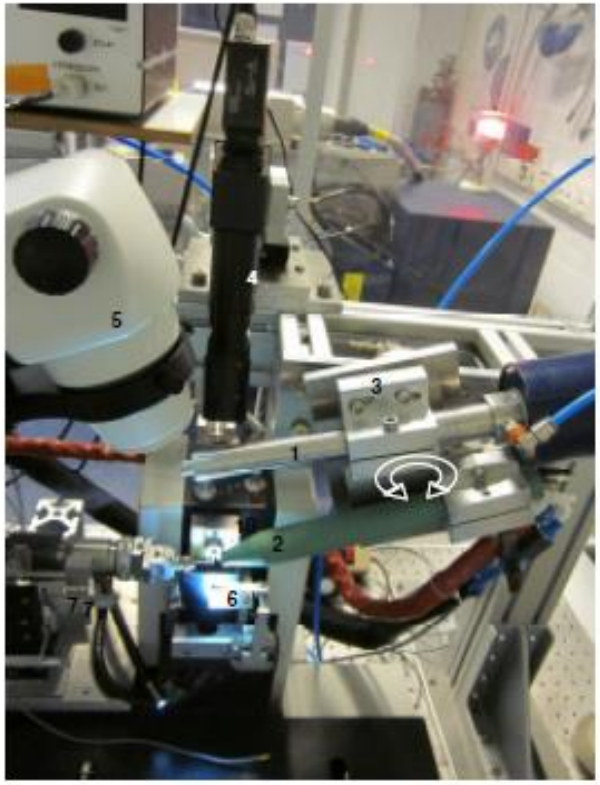


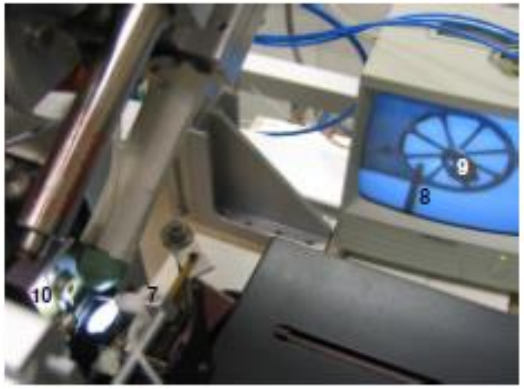


Science case: At the momement the Kappa geometry (to cover a larger part of the reciprocal space) is realized by tilting the crystal manually. With a mini-Kappa goniometer and a suitable strategy software an optimized data collection can be realized resulting in shorter data taking times.

Mini-kappa goniometer head for increasing the completeness of data sets, no need for manual unmounting and re-mounting of the crystal (reduced risk of loosing the crystal).

Humidity Controlled Gas Stream





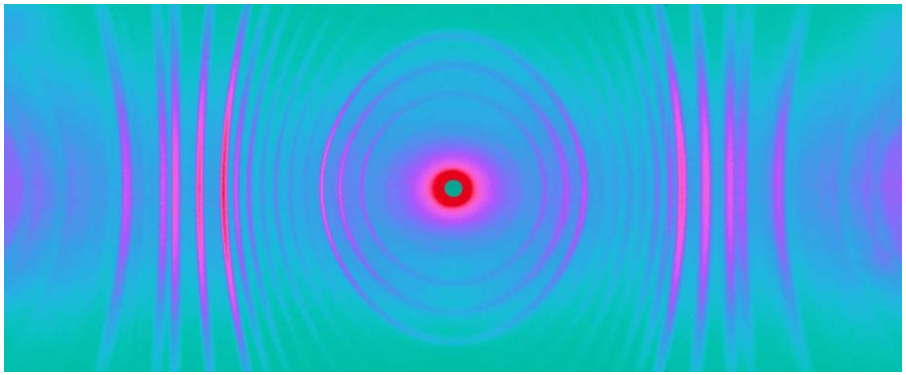
1 Cryo-nozzle
2 Humidifier-nozzle (FMS)
3 Rotational switch (pneumatic)
4 Video System with optics
5 Stereo Microscope
6 Light
7 Sucking device
8 Glass capillary
9 Crystal
10 Xray collimator

Picture taken from
http://www.proteros.de/

Science Case: 1) Improvement of crystal quality due to controlled hydration or dehydration with D2O vapour (already tested at Proteros in Martinsried)
2) Possible Contrast variation for low resolution studies (>4 Å): Lipids can be distinguished from protein content. Examples: Membrane proteins, virus proteins.

Summary

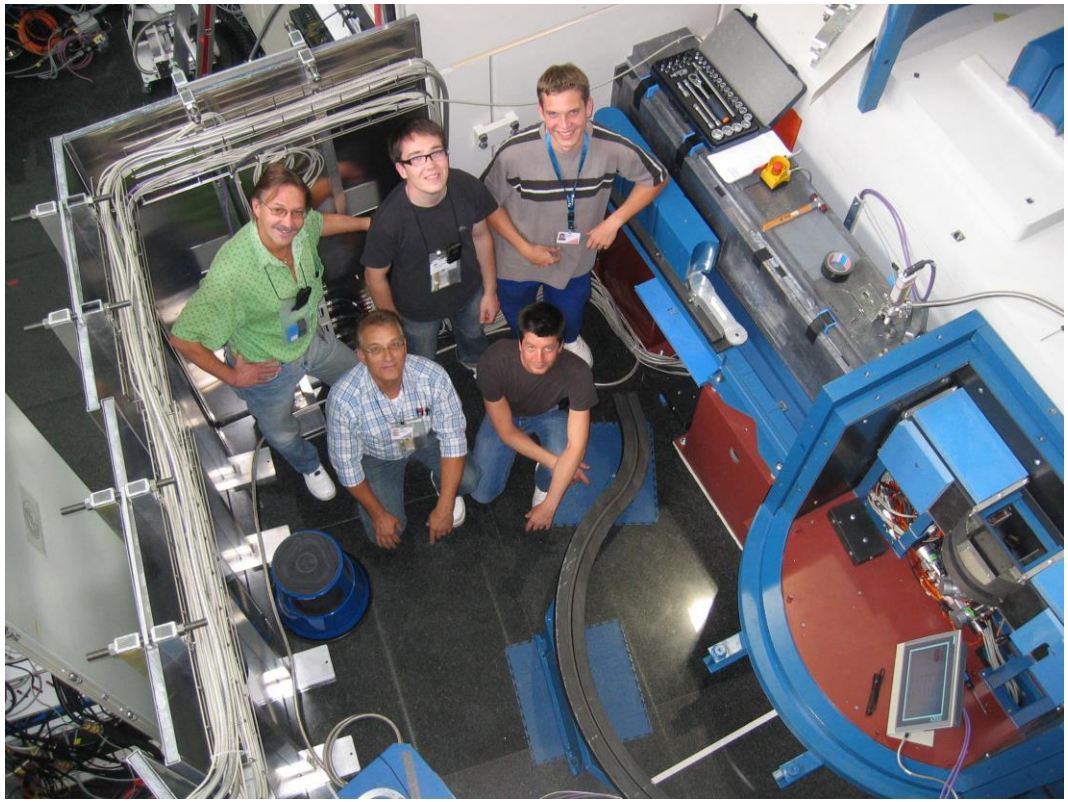
- The construction phase of the instrument BioDiff is finished.
- BioDiff is in normal user operation with a good over-booking factor.
- First user publications show that high quality data sets can be recorded.
- The applications of BioDiff have been extended beyond protein crystallography e.g . to determining magnetic unit cells in zero field, or investigate carbon dioxide adsorption in clay particles.
- Further improvements of the instrument will lead to broader applications and improved data taking speeds.



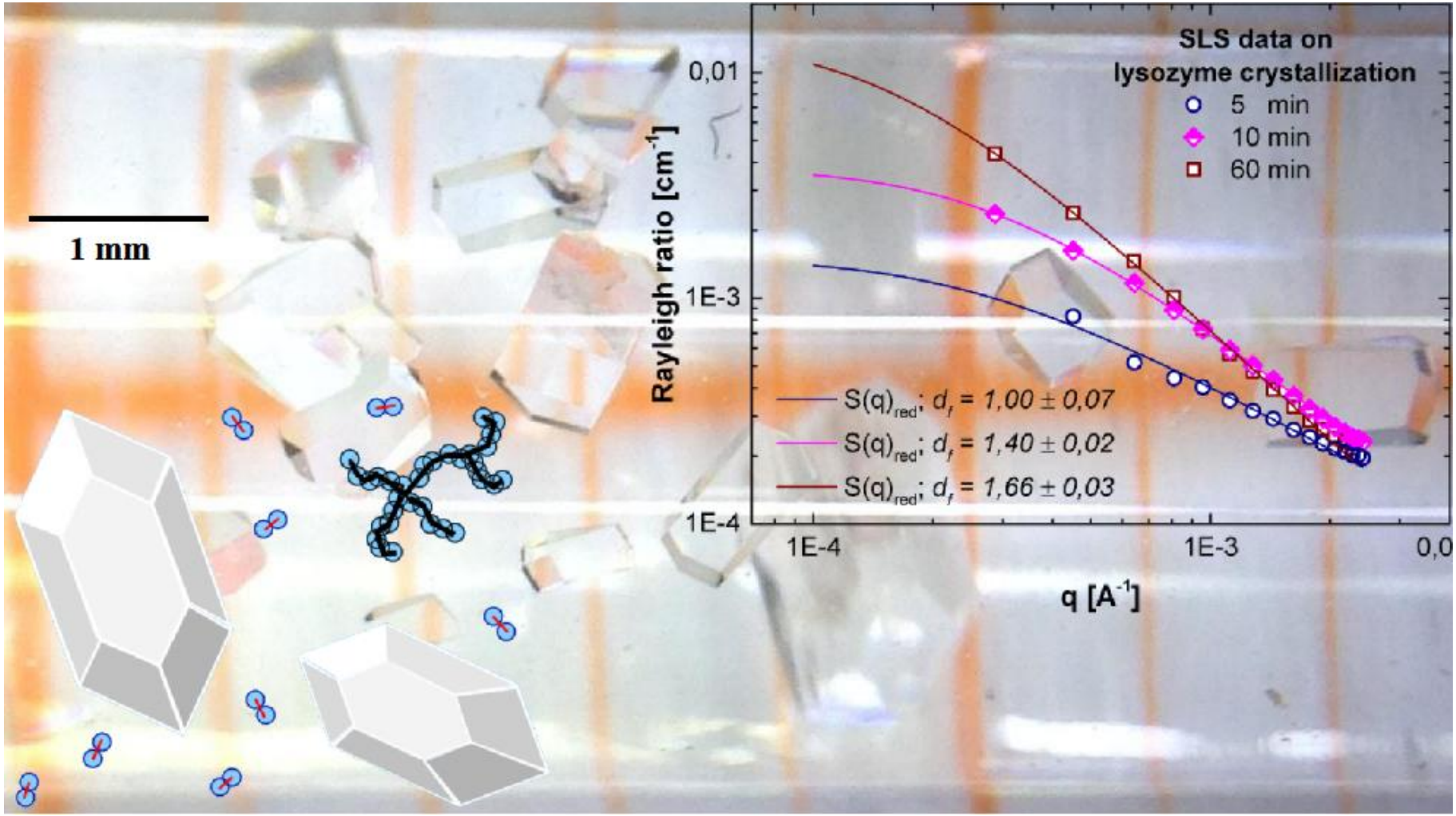
BioDiff as a powder diffractometer:
NAG powder sample
at 2.7 Å

Thanks to... ... the BioDiff-Team:

- Philipp Jüttner
- Reinhard Schätzler
- Bernhard Laatsch
- Frank Suxdorf
- Manfred Bednarek
- Matthias Drochner
- Harald Kleines
- Kevin Körrentz
- Karl-Heinz Mertens
- Michael Monkenbusch
- Nikolas Arendt
- Christian Felder
- Michael Wagener
- Lydia Fleischhauer-Fuss
- Vladimir Ossovyi
- Andreas Nebel
- Simon Staringer
- Harald Kusche
- Winfried Petry
- Dieter Richter



... and you for your attention!


1 mm
SLS data on
lysozyme crystallization
5 min
10 min
60 min
Rayleigh ratio [cm^{-1}]
0,01
1E-3
1E-4
S(q)$_{red}$; d_f = 1,00 ± 0,07
S(q)$_{red}$; d_f = 1,40 ± 0,02
S(q)$_{red}$; d_f = 1,66 ± 0,03
1E-4
1E-3
0,0
q [A^{-1}]

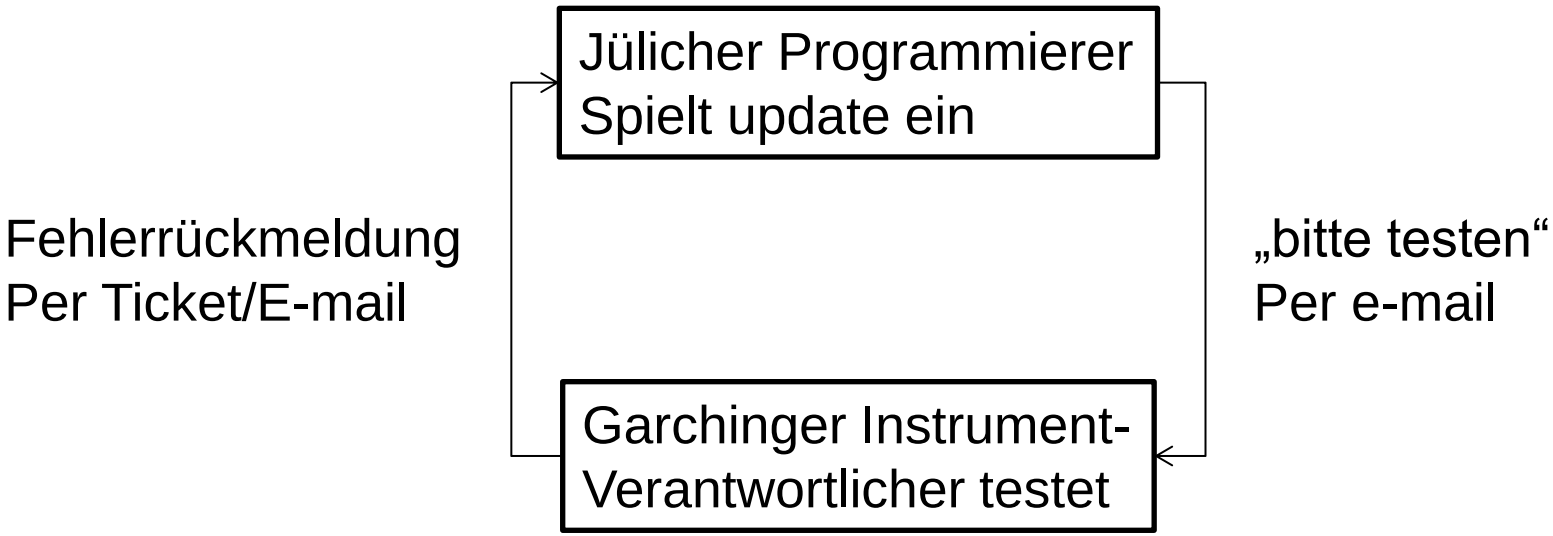
Tango-Workshop 4. - 6. März 2015

Man power situation: 16 programmers vs. 26 instruments

Harald Kleines
Nikolas Arendt
Jens Krüger
Alexander Lenz
Enrico Faulhaber
Stefan Huber
Pascal Neubert
Andreas Schulz
Stefan Rainow
Peter Kämmerling

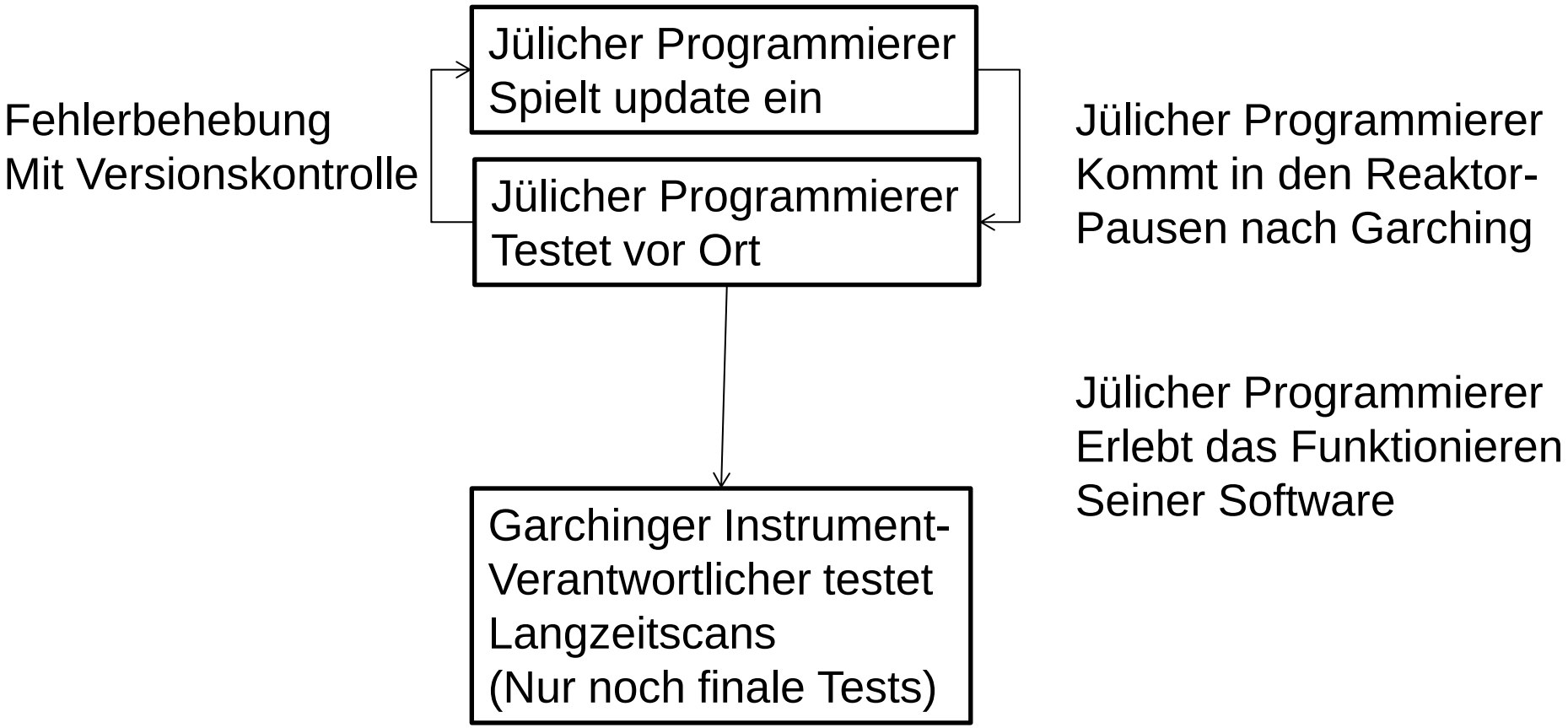
Michael Wagener
Stefanie Keuler
Franz Josef Kayser
Matthias Drochner
Georg Brandl
Christian Felder
Lydia Fleischhauer-Fuss
Josef Möller
Frank Suxdorf
Sven Janaschke

Open loop operation



Kein positives Feedback vom Benutzer

Closed loop operation



Weitere Vorschläge

1. Testkonzepte müssen implementiert werden (Testskripte, vor Ort Testtermine, klare Instruktion, was neu programmiert wurde)
2. Versionskontrolle (auch für SPS-Software)
3. Code-Styles, Code Reviewing
4. Vier-Augen oder mehr Prinzip: Es gibt immer noch einen weiteren, der über den Code drüberschaut und sich dann auch damit auskennt.
5. Dokumentation für den Anwender
6. Tango-Server verkaufen bzw. den Firmen anbieten, um eine gemeinsame Application Note zu schreiben
7. Eigene Veröffentlichungen zu Software-Lösungen schreiben

Kritische Punkte

1. ZEA-2 eigene Entwicklung einer Ethernet-Karte für ProfiNet
2. ISO 9001 Zertifizierung
3. Matrix-Struktur mit Resourcen-Management

Was ich mir von dem Workshop erhoffe:

Timing Verständnis der SPS/TANGO-server/Nicos Abläufe

Überblick über die Probleme an anderen Instrumenten (N. Arend

Einblick in die verwendeten Programmiertechniken

Ideen, wie man zuverlässige Nicos-kompatible Tango-server etwas standardisiert programmieren kann